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SDF-1 alone and in co-operation with HGF regulates biology of human cervical carcinoma cells

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Abstract: Stromal Derived Factor-1 (SDF-1)-CXCR4 axis plays a pivotal role in biology and metastasis of several tumors. The aim of this study was to see if SDF-1 alone or in combination with Hepatocyte Growth Factor (HGF) affects biology of human cervical carcinoma (HCC) cells. We found that HCC cell lines investigated in our study highly express CXCR4 on their surface. CXCR4 was also expressed on tumor cells in tissue sections derived from cervical cancer patients. At the same time normal cervical epithelium was negative for CXCR4 expression what suggests a strong correlation between CXCR4 and malignant cell phenotype. Subsequently, we studied a potential role of the SDF-1-CXCR4 axis in HCC and noticed that SDF-1 (i) chemoattracted HCC cells, (ii) enhanced their scattering, (iii) stimulated nuclear localization of β-catenins and upregulated their target gene cyclin D1 and (iv) at the molecular level induced calcium flux and activated RAS-MAPK, PI3-AKT and JAK-STAT pathways. SDF-1-mediated functions were additionally enhanced in the presence of HGF. Thus, our data show that the SDF-1-CXCR4 axis affects biology of HCC cells. Furthermore, we postulate that this axis might become a potential target to prevent progression of cervical cancer.

Key words: SDF-1 - CXCR4 - HGF - Metastasis - Cervical carcinoma - β-catenins

Introduction

Cervical carcinoma is a major cause of tumor-related deaths among women around the world and there are approximately five hundred thousand new incidents of this cancer each year worldwide [21, 23]. The cervical tumors vary in their invasiveness from non-metastatic tumors growing *in situ* to highly metastatic ones infiltrating neighboring tissues and distant organs [26]. Human cervical carcinoma metastasizes frequently to the lymph nodes, bones, liver and lungs. Thus, elucidation of mechanisms that lead to the spread of HCC may be important in developing antimetastatic therapeutic strategies.

Recently, SDF-1 - G protein-coupled seven transmembrane span receptor CXCR4 axis had been identified to play an important role in metastasis of several CXCR4-positive solid tumors such as breast cancer [18], ovarian cancer [24], lung cancer [13], prostate cancer [28], neuroblastoma [9], glioma [1] and rhabdomyosarcoma [12, 15]. Activation of CXCR4 on these tumors activates several processes that are essential for metastatic behavior of cancer cells (*e.g.*, motility, adhesion, secretion of metalloproteinases and angiopoietic factors). More importantly, CXCR4-positive tumor cells are chemoattracted and metastasize to organs that highly express SDF-1 (*e.g*, bones, lymph nodes, liver and lungs). However, no studies were reported yet on a potential role of the SDF-1-CXCR4 axis in regulating biology and metastasis of cervical carcinomas. The only relevant paper evaluated the activity of CXCR4 promoter in human cervical carcinoma cells. The authors found that this promoter possessed high activity in HCC cells [22].

In contrast to the SDF-1-CXCR4 axis, the role of HGF and its receptor MET in regulating metastatic behavior of HCC has been well established [25]. HGF

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that binds to tyrosine kinase receptor MET was found to stimulate proliferation and scattering of cervical carcinoma cells due to disruption of intercellular junctions, skeletal reorganization and enhancement of cell motility/migration [4, 25]. In our recent studies we noticed that SDF-1 and HGF may in a synergistic way influence the metastatic behavior of human rhabdomyosarcoma cells [12], suggesting that both factors may be involved in the spread of solid tumors that express CXCR4 and MET receptors.

In this study we asked if the SDF-1-CXCR4 axis alone or in synergy with HGF-MET receptor is involved in metastasis of HCC. To address this issue we phenotyped (i) established HCC, (ii) tumor samples obtained from patients suffering from metastatic cervical carcinoma, and (iii) cervical epithelium from normal healthy donors for CXCR4 expression. We also attempted to elucidate the role that the CXCR4-SDF-1 axis plays in cervical carcinoma tumor progression and its potential role in facilitating the metastatic behavior of HCC. Finally, we investigated if responsiveness of HCC to SDF-1 gradient is enhanced in the presence of HGF. Our data identified the CXCR4-SDF-1 axis as a potential target for developing new antimetastatic strategies in HCC.

Materials and methods

Cell lines and tissue samples. All the cervical carcinoma cell lines used in this study (HeLa, C33, HTB34, HTB35, CaSki, C4I) were purchased from ATCC (Rockville, USA) and maintained in DMEM (Gibco BRL) plus 10% FBS (Gibco BRL) at 37°C, 5% CO_2 and 95% humidity. Cervical carcinoma samples were derived from primary tumor site of patients with metastatic disease. Normal human cervical epithelium sample was taken during oncological resections of the cervix. After washing, the biopsy was placed in PBS containing dispase (12 U/mL) for 16 h at 4°C. Next, subendothelial tissue was removed and epithelium was treated with 0.05% trypsin for 10 min to isolate epithelial cells. The enzyme activity was eliminated by diluting with KGM-2 medium (Sigma). The cell suspension was then centrifuged for 5 min at 100 × g and the cell pellet was resuspended in KGM-2 serum-free medium. After 5-6 days, the epithelial cells were passaged and used for experiments. All experiments performed on human tissues were approved by the Institutional Board of Jagiellonian University Medical College, Cracow, Poland.

FACS analysis of CXCR4 expression. For FACS analysis cells were harvested using sodium versenate (SV) in order to prevent downregulation of the receptor expression after exposure to trypsin. The cells were incubated in the presence of SV for 10-20 min and subsequently washed with the culture medium. The cell pellet was resuspended in staining buffer (PBS + 5% FBS) at concentration of 10^7/ml. 100 µl of the cell suspension was used for staining. The CXCR4 antigen was detected with PE-anti-CXCR4 monoclonal antibody (MoAb) (R&D Systems), clone 12G5. The cells were incubated with 20 µl of antibody for 30 min at 4°C, washed twice in staining buffer and fixed with 1% paraformaldahyde before analysis. The FACS analysis was performed on FACSCalibur flow cytometer and analyzed with CellsQuest software (BDSciences).

Immunofluorescence staining of CXCR4. To demonstrate presence of CXCR4 in patients' samples, the immunofluorescence staining was performed using frozen tissues from metastatic tumors. Sections (3 µm) were cut in a cryostat and put on glass slides. Next, they were fixed with ice-cold acetone and washed in PBS. The sections were blocked with 0.5% BSA and incubated with mouse monoclonal antibody against human CXCR4, clone 12G5 at a concentration of 10 µg/ml (BDSciences) for 30 min at 37°C in a humid chamber. The sections were washed 3 times and stained with secondary anti-mouse antibody (1:200) conjugated with Alexa-488 (Molecular Probes) for 30 min at 37°C in a humid chamber. Next, they were rinsed twice with PBS, mounted and examined using Olympus BX51 fluorescence microscope equipped with a charge-coupled camera (Olympus America) and analyzed with Image-Pro software (Media Cybernetics, Inc.).

Chemotaxis studies. The chemotaxis towards SDF-1 and HGF gradient was studied using modified Boyden's chambers with 8-µm pore polycarbonate membrane inserts. The membrane was covered with 50 µl of 0.5% gelatin 10 min before the assay to facilitate cell attachment. Gelatin solution was discarded before the assay. Cells were detached with 0.25% trypsin, followed by inactivation with culture medium, resuspended in DMEM medium containing 0.5% BSA and seeded into the upper chamber of a Transwell insert (Costar Transwell) at a density of 5×10^4 per 100 µl. The lower chamber was filled with DMEM/BSA medium containing SDF and HGF at various concentrations. DMEM/BSA medium alone was used as a negative control. To determine whether the migration was stimulated by the gradient of the chemoattractant, in some samples SDF was also added into the upper chamber to equalize the difference in concentration between chambers. After 24 h, the inserts were removed from the transwells, cells remaining in the upper chamber were scraped off with cotton wool and the cells that had transmigrated were stained with Giemsa and counted under high power field (HPF). 5 fields were counted each time and the mean number of cells per HPF was calculated.

Proliferation assay. For the proliferation study cells were seeded in 96-well plates at 500 cell/well concentration in the culture medium. After attachment of cells, the medium was changed and replaced with DMEM containing 0.5% BSA and SDF-1 (10 ng/ml or 100 ng/ml), HGF (25 ng/ml, 2.5 ng/ml, or 0.25 ng/ml) or combination of both. Each assay was run in quadruplicate. After 72 h, 20 µl of the CellTiter96 Aqueous One Solution Cell Proliferation Assay (Promega) was added into each well. The plates were developed for 1-4 h, read using the ELx800 Universal Microplate Reader (Bio-tech) and analyzed using KC4 v3.0 with PowerReports software.

Western blots. Western blots were carried out on extracts prepared from CC cell lines (1×10^7 cells) which were seeded in 6-well plates. After cells adhered to the plastic, medium was changed and cells were kept in DMEM medium containing 0.5% BSA to render them quiescent. The cells were then stimulated with 100 ng/ml SDF-1β and 10 ng/ml HGF for 2, 10, 30, 60 and 120 min at 37°C, and lysed on ice in MPER lysing buffer (Pierce) containing protease and phosphatase inhibitors (Sigma). Subsequently, the extracted proteins were separated on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (Schleicher & Schuell) as previously described [15]. Phosphorylation of the intracellular kinases, 44/42 MAPK (Thr 202/Tyr 204) and AKT, as well as STAT5 proteins was detected using commercial mouse phospho-specific monoclonal antibodies (p44/42) or rabbit phospho-specific polyclonal antibodies (all from Cell Signaling) followed by HRP-conjugated goat anti-mouse IgG or goat antirabbit IgG as secondary antibodies (Santa Cruz Biotech), as described previously [15]. Equal loading in the lanes was evaluated by stripping the blots and re-probing with appropriate Abs against total proteins. The membranes were developed with an ECL reagent (Amersham Life Sciences), dried and subsequently exposed to HyperFilm (Amersham).

Calcium mobilization. Cells were incubated for 30 min at 30°C with 1-2 µM Fura-2/AM (Molecular Probes). After incubation, the cells were washed once and resuspended in loading buffer without FBS. Within 1

h cells were stimulated with SDF-1β (10 ng/ml), HGF (10 ng/ml) or both and analyzed using F-2500 fluorescence spectrophotometer (Hitachi).

Fluorescent staining of actin cytoskeleton and β-catenin. For the visualization of the actin cytoskeleton and β-catenin, cells were cultured for 12 h on glass coverslips in DMEM medium supplemented with 10% fetal bovine serum. Subsequently the medium was changed to DMEM/0.5% BSA and cells were cultured for next 12-24 h in the presence of 100 ng/ml SDF-1β, 10 ng/ml HGF or both. Next the cells were fixed in 3.7% paraformaldehyde/Ca- and Mg-free PBS for 15 min and permeabilized with 0.1% triton X-100 in PBS for 1 min at RT. For actin visualization cells were stained with Alexa488-phalloidin (Molecular Probes) at a concentration of 0.5 U/ml for 1 h. For β-catenin demonstration cells were stained with anti-β-catenin primary mouse antibody (sc-7963; Santa Cruz) at 1:100 dilution followed by secondary anti-mouse Alexa488-conjugated antibody (Molecular Probes) diluted 1:200. The stained cells were examined using Olympus BX51 fluorescence microscope equipped with a charge-coupled camera and analyzed with Image-Pro software.

RNA extraction and reverse transcription. Total RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany) followed by DNAse treatment (Promega, Madison, VI). The reverse polymerase transcription was performed using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA).

Quantitative real time PCR analysis. Cyclin D1 expression was determined by quantitative real time PCR analysis on the 7300 Real Time PCR System (Applied Biosystem) using a commercially available SYBR Green PCR Master Mix (Applied Biosystem) containing AmpliTaq Gold DNA Polymerase and SYBR Green I dye. Cyclin D1 was amplified using forward primer 5'-GCA TGT TCG TGG CCT CTA AGA T and reverse primer 5'-GAG AAG CTG TGC ATC TAC ACC G. Cells were seeded at low density, synchronized for 12 h in 0.5% BSA containing medium and stimulated for 24 h with SDF-1 (100 ng/ml) and HGF (10 ng/ml) in 0 .5% BSA medium. The mRNA expression level for all samples was normalized to the housekeeping β-actin gene amplified with forward primer 5'-GGA TGC AGA AGG AGA TCA CTA and reverse primer 5'-CGA TCC ACA CGG AGT ACT TG.

Statistics. Arithmetic means and standard deviations were calculated using Instat 1.14 (GraphPad) software. Data were analyzed using the Student t-test for unpaired samples.

Results

The expression of CXCR4 receptor on HCC cell lines

CXCR4 is expressed on several tumors. To address if this receptor is also expressed in HCC, six established human cervical carcinoma cell lines were evaluated for CXCR4 expression. We found that all cells lines evaluated in our study expressed CXCR4 by FACS (Fig. 1A). At the same time, however, no CXCR4 expression was found on normal cervical epithelium (Fig. 1B). The latter cells, however, highly expressed CD29 on their surface.

Next, the immunofluorescent staining for CXCR4 expression was performed on tissue samples derived from three patients with cervical cancer. We noticed that all samples derived from HCC samples showed strong immunostaining for CXCR4 (example in Fig. 1C).

SDF-1 stimulates migration of HeLa and C33a cells

The SDF-1-CXCR4 axis was reported to regulate metastatic behavior of several tumors. We became interested if this axis is also involved in metastasis of HCC and to assess a role of SDF-1 in regulating the metastatic behavior of HCC cells, we selected the HeLa cell line as a model. This cell line highly expresses CXCR4 (Fig. 1A) in addition to MET receptor (not shown) which binds HGF that is a known chemoattractant for HeLa cells. We found that HeLa cells were chemoattracted by a low concentration of both, SDF-1 (10 ng/ml) and HGF (10 ng/ml). Furthermore, when we employed both factors together an additive effect was observed (Fig. 2A). Similar results were obtained with another HCC cell line C33a (Fig. 2B). In contrast, SDF-1 at higher concentration (100 ng/ml) did not chemoattract HCC cells (not shown).

CXCR4 is functional on HeLa cells

We performed several assays to study if CXCR4 is functional on HeLa cells evoking such effects as calcium flux and phosphorylation of intracellular signaling pathways. Activation of calcium flux in cells is related to cell migration/adhesion and as reported, stimulation of CXCR4 by SDF-1 increases calcium flux in several cell types [16, 17]. Thus, we became interested if HeLa cells will respond to SDF-1 by calcium flux. Figure 3 shows that SDF-1 induces calcium flux in these cells. Furthermore, HGF alone did not induce calcium flux in HeLa cells, however, it slightly sensitized response of these cells to stimulation by SDF-1 - as evidenced by an enhanced calcium flux if SDF-1 and HGF were employed together (Fig. 3).

Subsequently, we evaluated in HeLa cells the effect of SDF-1 stimulation on phosphorylation of signaling pathways that are relevant for cell proliferation/survival and motility (RAS-MAPK, PI3-AKT, JAK-STAT). We noticed that SDF-1, similarly to HGF stimulated in HeLa cells phosphorylation of MAPK and AKT. Furthermore, SDF-1, but not HGF, activated STAT3 (Fig. 4A).

Since the duration of activation of intracellular pathways is an important factor in facilitating biological responses, we focused on the timing of SDF-1 stimulation (Fig. 4B). We found that MAPK stimulation was strongest at 2 and 10 min after SDF-1 addition and returned back to the baseline by 30 min. In contrast, phosphorylation of STAT-3 persisted for 60 min and returned to baseline only after 120 min (Fig. 4B).

SDF-1 does not stimulate proliferation of HeLa cells

Since HeLa cells responded strongly to SDF-1 by activation of various intracellular pathways including mi-

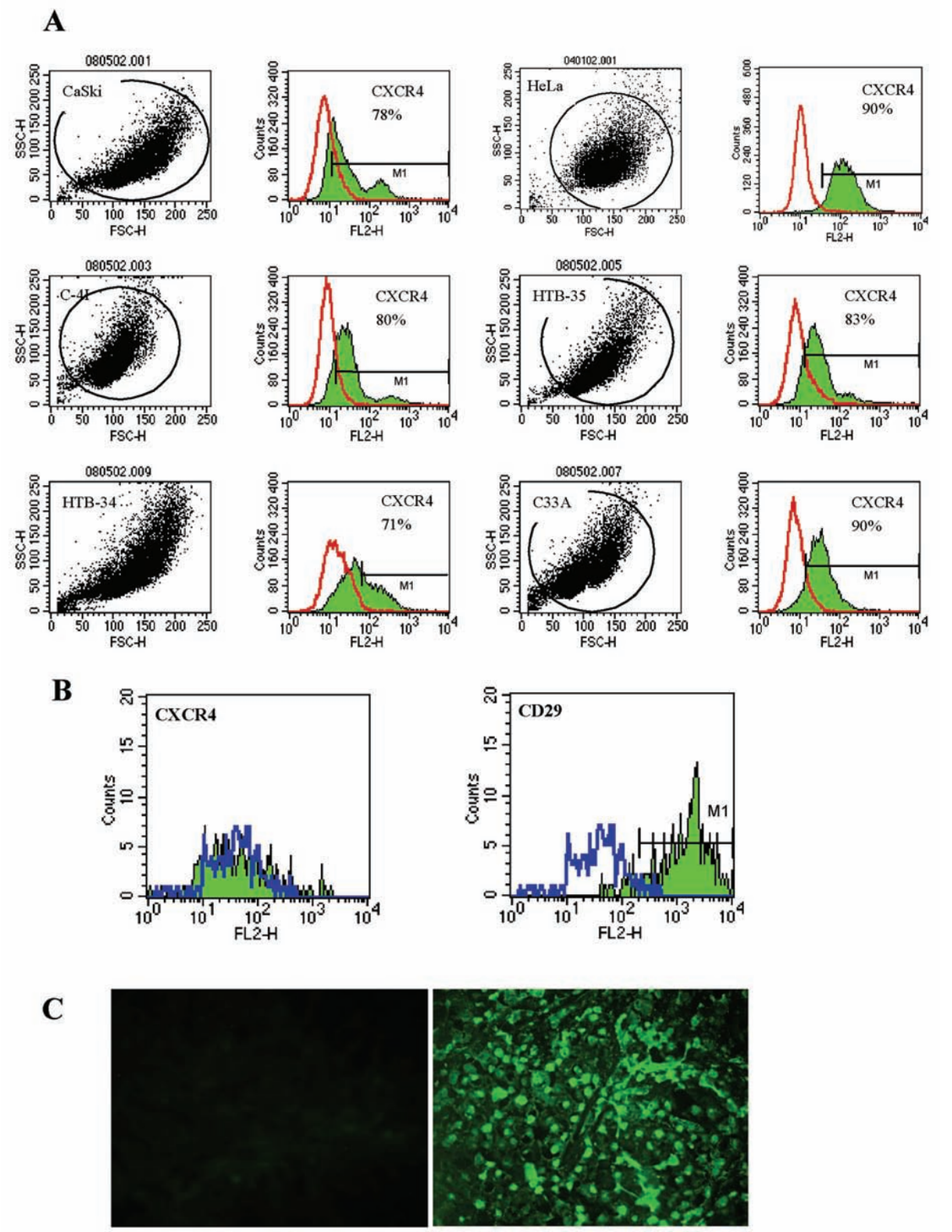

Fig. 1. Expression of CXCR4 on HCC cell lines, normal cervical epithelium and metastatic HCC sample. **A.** Flow cytometric analysis of six HCC cell lines stained for expression of CXCR4 using 12G5 monoclonal antibodies. All cell lines expressed CXCR4 in a range between 70% to 90%. **B.** Normal cervical epithelium does not express CXCR4. CD29 was used as a positive control. Cytometric analysis was performed using FACS Calibur. **C.** CXCR4 immunofluorescent staining of metastatic HCC tumor cells. Samples obtained from HCC patients contained strongly positive cells.

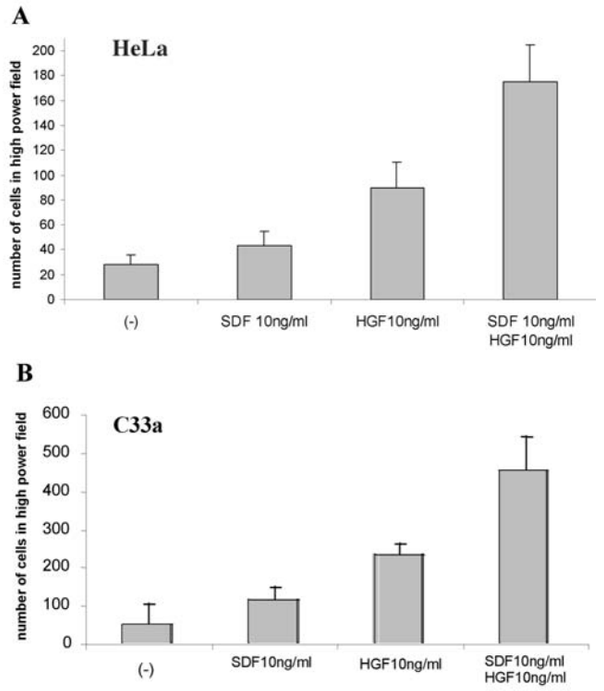

Fig. 2. SDF-1 enhances chemotaxis of cervical carcinoma cell lines toward an HGF gradient. HCC showed weak chemotaxis in the presence of low doses of SDF-1 (10 ng/ml). However, addition of SDF-1 at low concentration to medium containing 10 ng/ml HGF resulted in increased chemotaxis of HCC cells. Five high power fields (HPF) were counted and the mean number of cells per HPF was calculated.

togenic RAS-MAPK, we evaluated if SDF-1 can stimulate proliferation of these cells. The proliferation of HeLa cells was assessed under serum free conditions by employing MTT assay. SDF-1 was added at concentration of 10 ng/ml and 100 ng/ml and as a positive control, HGF was employed at concentration of 25 ng/ml, 2.5 ng/ml and 0.25 ng/ml, previously shown to stimulate proliferation of cervical carcinoma cells [5]. We did not observe, however, any effect of SDF-1 on cell proliferation, although HeLa cells proliferated in response to HGF (data not shown).

SDF-1 and HGF stimulate HCC cell scattering

HeLa cells are growing *in vitro* in close contact forming clusters visible under an inverted microscope (Fig. 5A). Since tumor cell scattering is important for invasion, we evaluated if SDF-1 and HGF induce scattering of these cells. We noticed that both SDF-1 and HGF increased scattering of HeLa cells and that this effect was dose dependent (Fig. 5 B-I).

F-actin reorganization after SDF-1 and HGF stimulation

Reorganization of F-actin is essential for cell migration/adhesion. We noticed that HeLa cells stimulated with SDF-1 (Fig. 6 B) spread from the clusters and, more importantly, F-actin tended to accumulate at one edge of the cell (Fig. 6). This type of F-actin organization and polarization of cell shape followed by functional asymmetry manifested by the formation of leading lamella is typical for migratory cells and is often referred to as the leading edge [30]. This effect was additionally enhanced in the presence of HGF (Fig. 6D).

Nuclear accumulation of β-catenins and target gene activation

Beta-catenin is an important component of adherent junctions and may translocate upon stimulation from the cell membrane into the nucleus, where it plays a role in

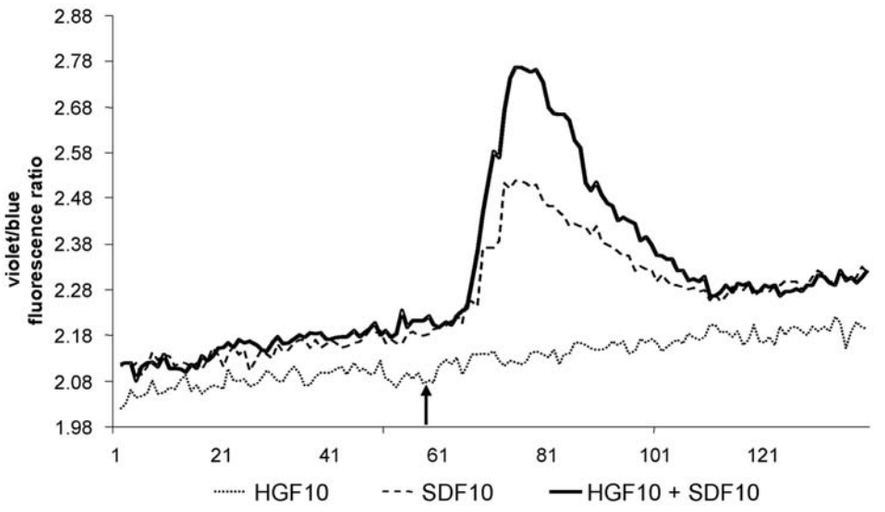

Fig. 3. SDF-1 induces calcium flux in HeLa cells. HeLa cells stimulated with 100 ng/ml of SDF-1 showed a strong increase in intracellular calcium levels. HGF/SF alone did not induce calcium flux in HeLa cells but it sensitized cells to stimulation with SDF-1 as observed by an enhanced calcium flux when both factors were employed together.

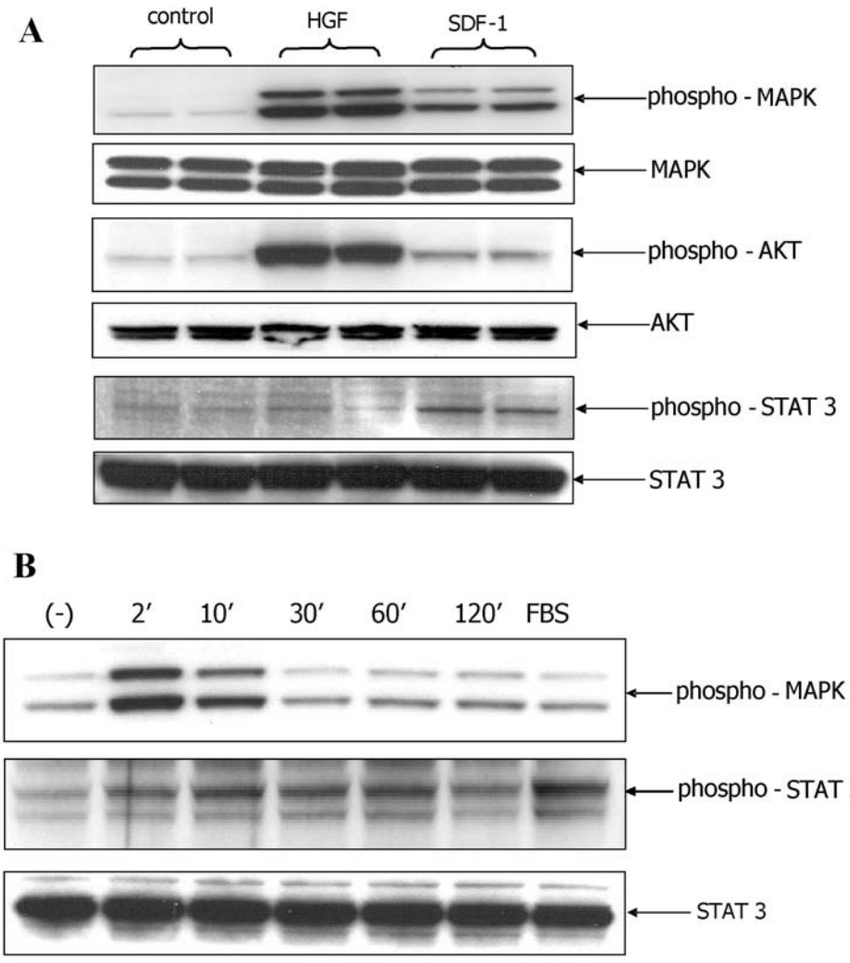

Fig. 4. SDF-1 activates intracellular signaling pathways. Data was obtained using western blot assay. **A.** Incubation of HCC cells with SDF-1 resulted in phosphorylation of MAPK p44/p42, AKT and STAT3 proteins. Activation of MAPK and AKT was lower in comparison to HGF but the level of STAT3 activation was higher. **B.** Time course of SDF-1 activation. MAPK phosphorylation peaked at 2 minutes, lasted out to 10 minutes and returned to a control level after 30 minutes. STAT3 activation was prolonged and was seen for 60 minutes.

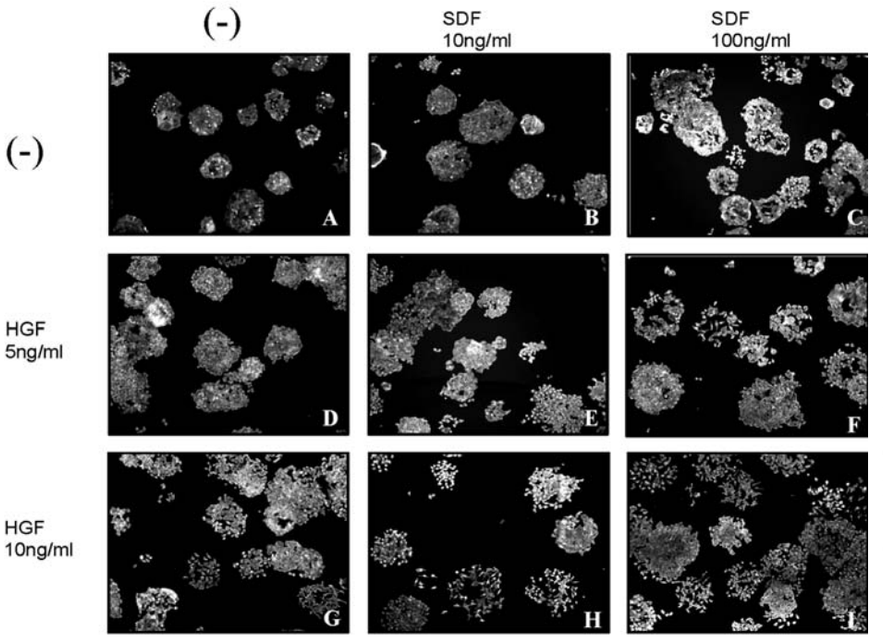

Fig. 5. SDF-1 enhances scattering effect of HGF. HCC cells growing in clusters were stimulated with a low (10 ng/ml - **B**, **E**, **H**) and high concentration (100 ng/ml - C, F, I) of SDF-1 as well as low (5 ng/ml - **D**, **E**, **F**) and high (10 ng/ml - **G**, **H**, **I**) concentration of HGF; **A** - unstimulated control. SDF-1 increases scattering of HCC induced by HGF in a dose dependent manner.

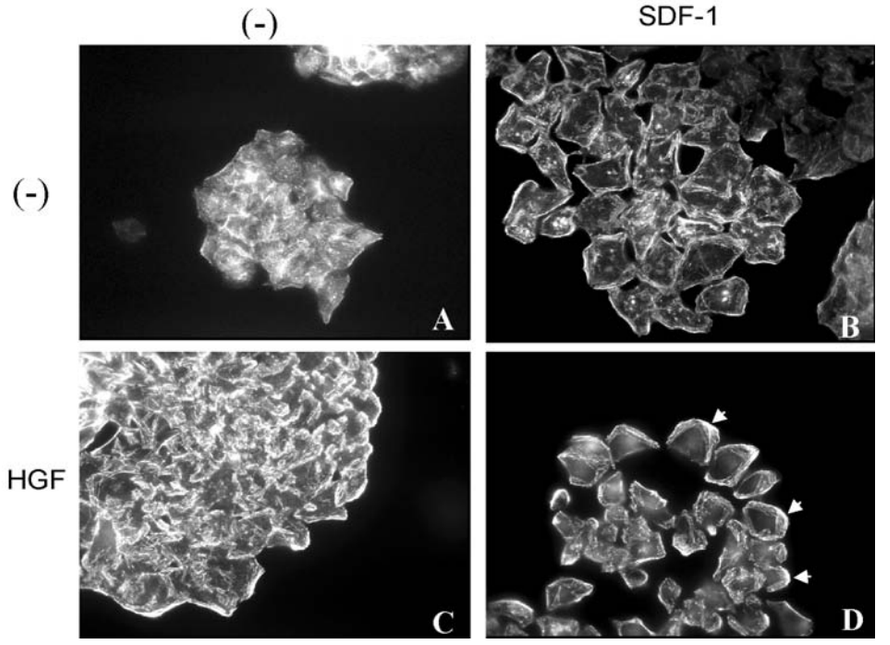

Fig. 6. SDF-1 together with HGF induces a migratory phenotype of HCC cells. Stimulation of HCC cells with SDF-1 (100 ng/ml - **B**, **D**) and HGF (10 ng/ml - **C**, **D**) induces spreading of HCC from clusters and reorganization of F-actin that tends to accumulate at the leading edge (arrowheads); **A** - unstimulated control. F-actin visualized using Alexa488-phalloidin staining.

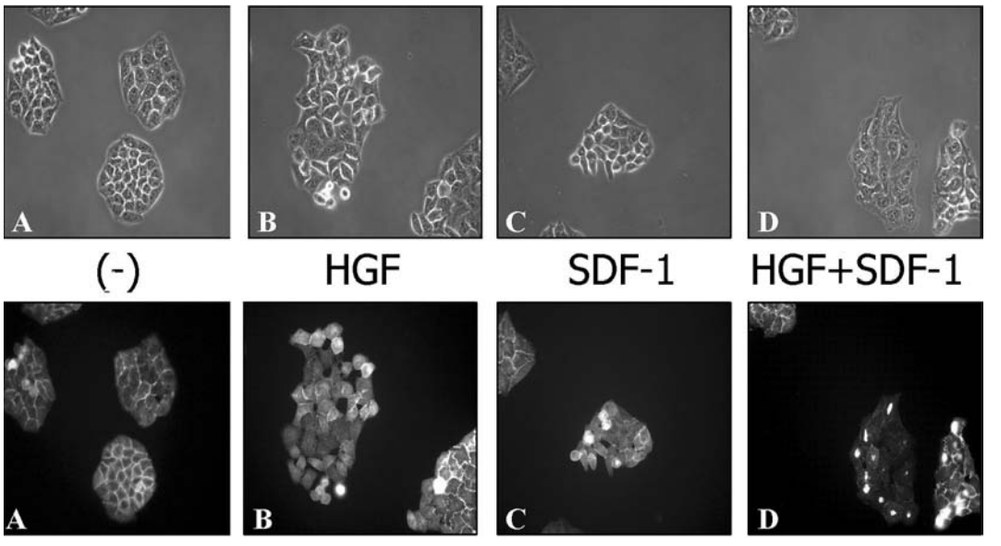

Fig. 7. Disruption of cell junctions and nuclear translocation of β-catenins after stimulation with both SDF-1 and HGF. HCC cells cultured in the presence of SDF-1 (100 ng/ml) and HGF (10 ng/ml) show diminished expression of β-catenins in the cell-cell junctions and its accumulation in the cell nuclei. Upper panel - phase contrast microscope images; lower panel - fluorescence microscope images.

the activation of several genes [2, 3]. Thus, we investigated the influence of SDF-1 stimulation on the pattern of β-catenin expression in HCC cells (Fig. 7). In unstimulated HeLa cells, the β-catenin staining was localized to the cell membrane (Fig. 7A). When HGF was used, the staining became more diffuse, indicating that the cells were losing cell to cell contact (Fig. 7B). Similar effects were observed after SDF-1 stimulation (Fig. 7C). More importantly, when both ligands were added simultaneously, β-catenins translocated to the nucleus as evidenced by their nuclear accumulation (Fig. 7D).

To study if translocation of β-catenins leads to the activation of Tcl/Lef transcription factor and subsequently to upregulation of its target genes we evaluated the effect of

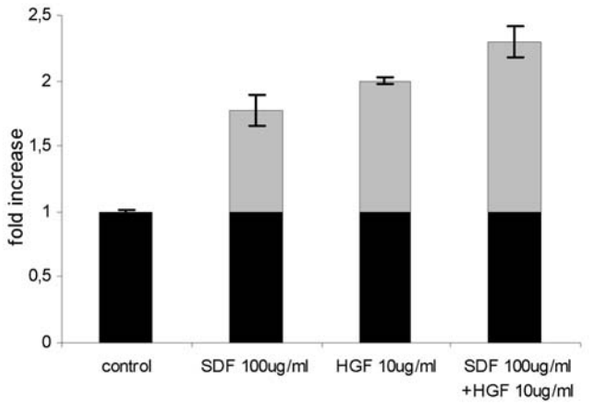

Fig. 8. HCC cells upregulate cyclin D1 mRNA expression after SDF-1 and HGF stimulation. HCC cells were cultured in the presence or absence of SDF-1 and HGF and cyclin D1 expression was measured by qRT-PCR. Black parts of bars represent levels of cyclin D1 expression in unstimulated cells. Gray parts of bars show the increase in comparison to control. Experiment was repeated twice with similar results.

SDF-1 stimulation on expression of cyclin D1 that is one the known β-catenins-Tcl/Lef target genes. We found that after 24 hours cyclin D1 expression was increased almost two times following stimulation by SDF-1 and/or HGF (Fig. 8).

Discussion

Cervical tumors differ in their tumorigenicity, from benign non-invasive to highly malignant invading the neighbouring tissues [26]. The role of some growth factors and cytokines in the invasive phenotype of HCC has already been established [32, 33]. As an example, HGF was shown to induce the invasive phenotype in HCC [25]. Invasion of adjacent tissues is characterised by disruption of cell-cell junctions followed by enhanced cell motility and HGF was shown to enhanced HCC scattering and motility.

Recently, a lot of attention has been focused on the role of chemokines and chemokine receptors in dissemination and metastasis of solid tumors [12, 15, 18]. In particular, the role of the SDF-1-CXCR4 axis in tumor invasion has been studied. It has been shown that expression of CXCR4 on breast cancer cells is one of important factors affecting their metastasis to the bone marrow [18]. There are also reports that SDF-1-CXCR4 may play a role in lymph node metastasis of oral epithelium squamous cell carcinoma [31] and squamous cell carcinoma of the tongue [6]. Our group reported recently that SDF-1 together with HGF could play a very important role in invasion of rhabdomyosarcoma cells to the bone marrow [12, 15].

In this paper, we studied the expression of CXCR4 on HCC-derived cell lines as well as on primary tissue samples from patients suffering from cervical carcinoma. We also looked at the biological role SDF and its receptor CXCR4 may play alone or in combination with HGF in proliferation, chemotaxis and cytoskeletal reorganization in HCC cell lines. All of these events are crucial for tumor metastasis and progression.

We noticed that both HCC-derived cell lines and tissue from HCC patients express CXCR4. At the same time CXCR4 was absent from normal epithelial cells. This suggests that CXCR4 expression correlates with cervical epithelium malignancy. SDF-1 was shown to stimulate migration of normal and malignant cells [14, 15, 18]. Since the invasion is dependent on cell sensitivity to the chemokine gradient, we studied if SDF-1 can stimulate chemotaxis of HCC cell lines. We found that SDF-1 is able to stimulate migration of HCC cell lines if employed at low doses, however, this effect was absent when higher concentrations of SDF-1 were employed. This observation is in agreement with previous reports showing dose-dependent desensitization of chemokine receptors [11]. Furthermore, chemotaxis correlates with an intracellular increase in calcium concentration that is stimulated by chemokines. Our calcium flux experiments confirmed that SDF-1 is able to stimulate calcium mobilization in HCC cells. This effect was enhanced in the presence of HGF/SF. Similar data were reported previously in haematopoietic cells. Groopman *et al*. [8] observed an increase in calcium flux in hematopoietic CT cells when SDF-1 and stem cell factor were used simultaneously. Our data clearly show that SDF-1 is a chemoattractant for HCC cells and that SDF-1 together with HGF promotes cervical carcinoma cell scattering.

We also demonstrated F-actin reorganization following stimulation with both SDF-1 and HGF. We observed accumulation of F-actin in the region of the leading edge of stimulated cells. This type of change in intracellular distribution of F-actin is characteristic for cells acquiring an invasive phenotype. This enables the cells to move by firmly attaching to the surface at the leading edge while contracting the lagging edge [30]. To our surprise, we also noticed changes in the surface distribution of β-catenins and their nuclear accumulation in cells simultaneously stimulated by SDF-1 and HGF.

Beta-catenins play a very important role in the integrity of adherent junctions and are responsible for connecting E-cadherins to cytoskeletal proteins such as F-actin [3]. After nuclear translocation β-catenins may also act as transcription factors and together with the Tcl/Lef family of transcription factors activate several genes - c-myc, cyclin D and MDR - which are responsible for tumor progression [2, 10]. Indeed, in our study we observed upregulation of mRNA expression for cyclin D1 which suggests that β-catenins-Tcl/Lef complex is active and able to stimulate expression of its target genes. Although we did not observe a direct effect of

SDF-1 on cell proliferation in this study, an activation of cyclin D1 after SDF-1 stimulation confirms that SDF-1 in fact can enhance cell proliferation in other experimental models [19, 27, 34]. In some colon tumors in which APC mutation occurs, the regulation of β-catenins is disrupted and they accumulate gradually in the nucleus [20]. This nuclear accumulation of β-catenins is responsible for expansion of the tumors . Whether accumulation of β-catenins and activation of transcription complexes after stimulation with SDF-1 may be responsible for activation of other tumor-associated genes in HCC cells, is currently under investigation in our laboratory.

In this paper we have shown that CXCR4 receptor is functional and that binding of SDF-1 stimulates cell movement, cytoskeleton reorganization and gene activation. We have also demonstrated that SDF-1 can synergize with HGF in promoting tumor cell motility. The excessive tumor cell motility is a hallmark of the aggressive and metastatic tumor phenotype. We believe that the SDF-1-CXCR4 axis could be responsible for cervical carcinoma tumor invasion into adjacent tissue and subsequently into distant organs that secret SDF-1 such as bones, lymph nodes and liver. According to our results, we think that this axis could be a potential target for antitumor therapies. Several small molecule inhibitors of this axis have been already developed [7, 29] and could be used in preventing the dissemination of cervical carcinoma cells. This possibility is also currently investigated in our laboratory.

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